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Diabetes & Obesity International Journal
ISSN: 2574-7770

A Study on the Association of Age, Gender and ACE I/D Polymorphism with Cardiovascular Risk Factors (CRFs) among Urban Population of Chandigarh, India

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Research Article
Volume 6 Issue 1
Received Date: August 12, 2021
Published Date: September 22, 2021
DOI: 10.23880/doij-16000248

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Abstract

Cardiovascular diseases (CVDs) are multifactorial and predisposed by several risk factors. CVDs prevalence is attributed to cardiovascular risk factors (CRFs) such as hypertension, obesity, diabetes, ageing, gender and genetics. The specificity of CRFs to the habitat and lifestyle modification has differential CVDs outcomes. In the present cross-sectional study, we aimed at finding the prevalence of CRFs in an urban population. We also assessed the role of ACE I/D polymorphism, age and gender in CRFs susceptibility. Forty-four adult subjects, permanent residents of Chandigarh, India, were recruited in the present study. Anthropometric, physiological and demographic data were collected and analyzed. More than half of the subjects were having obesity and high blood pressure. Hyperglycemia was present in 43.9% of the subjects. Among ACE I/D genotypes, DD (45.5%) was followed by II (31.8%) and ID (22.7%), with a significant deviation from Hardy Weinberg Equilibrium. The high trends of CRFs depict that cardiovascular disease risk is very high in the present population of Chandigarh, India. The age might be one factor for this surge as subjects >45 years displayed enhancement in combined CRFs. Urbanization might be another possible reason for CRFs surge. Gender and ACE I/D polymorphism was not associated with CRFs in the present study. Though their trend exhibited a possible association, the ACE DD genotype has the lowest frequency among combined CRFs and females have a high frequency of CRFs. The most critical point of this study is the alarming/exponential increase in CRFs in Chandigarh. A study with bigger sample size is warranted regarding CRFs to identify the reasons for their surge in Chandigarh, India.

Keywords: Cardiovascular Risk Factors; Ageing; Gender; ACE I/D Polymorphism; Urbanization

Abbreviations: CVDs: Cardiovascular Diseases; CRFs: Cardiovascular Risk Factors; SBP: Systolic Blood Pressure; DBP: The Diastolic Blood Pressure; MAP: Mean Arterial Pressure

Introduction

Cardiovascular system (CVDs) diseases are the commonest cause of morbidity and mortality in developed

and developing countries despite advances in medical management [1-3]. CVDs are multifactorial, and associated co-factors like dyslipidemia, hypertension, diabetes, obesity, smoking, gender, and physical inactivity make individuals more prone to these diseases [1]. Of all cardiovascular risk factors (CRFs), hypertension, obesity and diabetes are the highest contributors to CVDs risk, and their prevalence is on the surge globally [4-8].

In developing countries, hypertension is a significant contributor to cardiovascular and renal diseases burden [9]. Ageing, obesity, harmful alcohol intake, familial hypertension, and diabetes are independently related to hypertension [9,10]. Similarly, the proportions of people with type 2 diabetes and obesity have progressively increased throughout Asia [5]. From 1980 to 2014, diabetes prevalence has increased to quadrupled [6]. In India, obesity is prevalent in more than 135 million people. According to the ICMR-INDIAB study 2015, the prevalence rate of obesity varies from 11.8% to 31.3% [8]. Obesity, independently and in association with several other risk factors, is closely connected with CVDs [1]. The combined risk of CVDs associated co-factors is more when compared to discrete effects [11].

The prevalence of CVDs associated risk factors is governed by age and gender more profoundly than other variables. Hypertension is the most common problem in middle and older age, leading to cognitive decline and cardiovascular morbidity and mortality [12-14]. Further, the pathophysiological basis of cardiovascular health among men and women is also not identical [4,15-17]. The prevalence of obesity rises steadily among older age groups and women [18,19]. Obesity and diabetes have been shown to alter glucose and protein metabolism in older age, especially in women. The gender differences in the prevalence of CVDs risk factors were observed in hypertension [4,15,16,20,21], obesity [5,8,22] and diabetes [22-24].

Cardiovascular diseases are multifactorial, and being a non-communicable disease predisposed by several risk factors, genetics is one of them. The genetic predisposition of CVDs associated co-factors is studied extensively in the past. The genetic variant more or less confound for CVDs progression through altering co-factors. A 21kb ACE gene is located on chromosome 17q23, consisting of 26 exons, 25 introns confound through it's I/D polymorphism. The Ace gene I/D polymorphism results from the insertion(I) or deletion(D) of 287 bp Alu sequence near the 3' end of intron 16 and form three genotypes DD, II and ID. ACE gene I/D polymorphism has been associated with high insulin resistance, a potential risk for type 2 diabetes [25,26] and overweight / obesity susceptibility [27]. The ACE genetic variation may modify the effect of obesity on hypertension in adulthood [28]. ACE gene I/D polymorphism is independently associated with coronary heart disease [29]. However, some studies could not find any associations of hypertension, obesity and diabetes with ACE I/D polymorphism [30-32].

Based on the above-listed age and gender-specific effects of hypertension, obesity, and diabetes on cardiovascular disease enhancement, the present study aims to identify the prevalence of these risk factors in an urban population. Further, the inconsistent results of genetic susceptibility in these risk factors, especially with ACE I/D polymorphism, form the basis of the present study. In the present study, we assess the role of ACE I/D polymorphism, age and gender in CVDs risk factors susceptibility.

Methodology

Subjects

The present study is a cross-sectional study carried out in the Union Territory of Chandigarh, India. In a general Health check-up, the subjects were examined physically, and their medical history was recorded. Forty-four subjects (with mixed caste) of the adult age group, permanent residents of Chandigarh, were recruited in the present study. Informed consent was taken from all subjects before data collection.

Data Collection

Data including age, sex, marital status, height, weight were collected for analysis. BMI was calculated and categorized according to the following formula based on the WHO scale of adult's BMI of the Asian population [33].

BMI= Body weight (in kgs.)/ Square of Height (in meters)
Normal = 18.4 - 23.9
Underweight =<18.4
Overweight = 23.9- 25.0
Obese = >25.0

Blood pressure was measured from the left arm in a sitting position after 10 minutes of resting by an automated blood pressure monitor (Dr Morepen BP02). Blood pressure was categorized according to the JNC VII scale [3] as:

<120/80 = Normal
120-139/80-89= Prehypertension
>139/>89= Hypertension

Normal and High Blood pressure (Pre-Hypertensive + Hypertensive) were used in our data analysis.

The postprandial blood glucose was measured using the automated strip based blood glucose monitor (Abbott Freestyle Optimum Neo). The data were categorized for blood glucose levels as normal (<110), hyperglycemic (>110)

[34]. 6ml of venous blood was taken in two tubes (red top and EDTA) in check with proper blood collection kits and sterilized conditions.

Laboratory Analysis

Laboratory analysis was done at Origin LIFE Healthcare Solution & Research Centre, Chandigarh. Out of 6ml of venous blood, 4ml of blood was taken in a red top serum tube for biochemistry analysis & 2ml of blood in an EDTA tube for genetic study. 4ml of venous blood containing a red top tube was subjected to serum separation. Serum was collected in a 2ml round cap tube and stored at 4^0C for further analysis. 2ml of venous blood in the EDTA tube was subjected to DNA extraction with the help of the Phenol Chloroform method [35].

Genetic Analysis

The extracted DNA further subjected for PCR amplification for ACE I/D polymorphism (rs4646994) [36]. The primers used were: F:5'-CTGGAGACCACTCCCATCCTTTCT–3' and R:5'–GATGTGGCCATCACATTCGTCAGAT – 3'. The reaction was carried out with 20pmol of each primer in a final volume of 10µl containing 10ng of genomic DNA, 10x Taq PCR buffer, 25mM MgCl2, 100mM of each dNTPs and 5U/uL of Taq polymerase. The genotyping was done using gel electrophoresis of PCR products on 2% agarose gel. The II genotype was identified as a 490 bp fragment, while a 190 bp fragment was observed in the DD genotype. In heterozygous ID genotype, both 490bp and 190bp fragments are present.

Statistical Analysis

The descriptive data were expressed in terms of mean, median and frequency as appropriate. Data were analyzed using IBM SPSS (Statistical Package for Social Science, 26). Genotype and allele frequencies were calculated using POPGENE software. Chi-square analysis was done to see a deviation in genotypic distribution regarding Hardy-Weinberg equilibrium. The p-value <=0.05 was considered significant.

Result

General Characteristics

The general descriptive characteristics of the subjects were recorded in Table 1. The mean age of the population is 46.73±13.73. The overall obesity is on the higher side as the mean value of BMI is 26.07±4.15. The population is hypertensive for isolated systolic blood pressure (SBP). The diastolic blood pressure (DBP) and mean arterial pressure (MAP) are in the pre-hypertensive stage. Further, the population is pre-diabetic for postprandial blood sugar as the value exceeding the normal range but below the diabetic range.

Genotypic Distribution

The overall distribution of ACE I/D Genotype was recorded in Table 2. The frequency of DD genotype showed a higher frequency (45.5%), followed by II (31.8%) and ID (22.7%). However, the genotypic distribution in the population is not following Hardy-Weinberg equilibrium (p-value=0.0003). A similar trend was shown in gender-wise and age-wise distribution of genotypes, where DD genotype is more predominant compared to the other two. The difference in males and females for the distribution of genotypes is not significant (p-value = 0.220). Similarly, there is no significant difference (p-value = 0.297) in young and old age concerning the genotype distribution. Although, II genotype is more prevalent among females and ID genotype is more prevalent in males. ID genotype is prevalent in older age, whereas II and ID genotype is more prevalent in younger age.

Variables	Mean/Median/ Percent
Age	46.73 ± 13.73
BMI	26.07 ± 4.15
SBP	146.07 ± 22.11
DBP	84.53 ± 12.17
MAP	104.28 ±15.99
Blood Sugar (Random)	107 (92-137)
OBS (Overweight + Obese)	68.20%
HBP (Hypertension+pre-hypertension)	73.20%
Isolated Systolic HBP	56.10%
Isolated Diastolic HBP	58.30%
DIA	43.90%
DIA + HBP	35.90%
OBS + HBP	56.10%
OBS + DIA	29.30%
OBS + HBP + DIA	23.10%

Table 1: General characteristics of the population.
BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure**,** MAP**:** Mean Arterial Pressure, OBS: Obesity, HBP: High Blood Pressure, DIA: Diabetes

Genotype	OVERALL				GENDER					AGE				
	N	%	χ2	p-Value	FEMALES		MALES		p-Value	≤45		>45		p-Value
					N	%	N	%		N	%	N	%	
II	14	31.8	12.68	<0.001*	6	46.2	8	25.8	0.22	7	38.9	7	26.9	0.297
ID	10	22.7			1	7.7	9	29		2	11.1	8	30.8	
DD	20	45.5			6	46.3	14	45.2	0.403	9	50	11	42.3	
I Allele	38	43.2	10.8	<0.001*	13	50	25	40.4		16	44.5	22	42.4	0.842
D Allele	50	56.8			13	50	37	59.6		20	55.6	30	57.6	
II + ID	24	54.5	14.08	<0.001*	7	26.9	17	27.42	0.577	9	25	15	28.8	0.647
DD + ID	30	68.1			7	26.9	23	37.1		11	30.5	19	36.5	
DD + II	34	77.2			12	45.15	22	35.4		16	44.4	18	34.6	

Table 2: The frequency distribution of ACE I/D polymorphism.
*p-value – Significant (<0.05)

Cardiovascular Risk Factor (CRFs) Distribution

The frequency of all the CRFs considered in the present study showed a higher trend in the subjects. More than 50% of the subjects have hypertension and obesity, whereas almost 44% are hyperglycemic. The combined CRFs also showed a higher prevalence ranging from 23.1% - 56.1%. Further, when the CRFs are compared between males and females, their abnormal components show higher frequency in females. However, this difference is not statistically significant. Older subjects showed a higher prevalence of CRFs than younger age, suggesting a higher risk of these co-factors in that age group. However, none of these co-factors reaches a significant difference except the combination of Diabetes and Hypertension (p-value = 0.035) and a combination of Obesity, Diabetes and Hypertension, which is on the verge of significance (p-value = 0.077).

CRFs vs ACE I/D Polymorphism

The distribution of CRFs with ACE I/D polymorphism showed a mixed trend (Table 3). The individual with II genotype displayed the highest frequency of BMI, diabetes and obesity plus diabetes. ID genotype showed a higher frequency of hypertension, MAP and obesity plus Hypertension plus diabetes. DD genotype showed a higher frequency of obesity plus hypertension and the lowest frequency of diabetes plus hypertension, obesity plus diabetes and obesity plus hypertension plus diabetes. Although CRFs show a difference in distribution, none of these factors reached the level of significance.

Variables	Gender					Age					ACE Genotype						
	Females		Males		p-Value	≤45		>45		p-Value	II		ID		DD		p-Value
	N	%	N	%		N	%	N	%		N	%	N	%	N	%	
BMI	11	84.6	19	61.3	0.13	11	61.1	19	73.1	0.402	10	71.4	6	60	14	70	0.816
HBP	10	76.9	20	71.4	0.712	9	60	21	80.8	0.148	9	64.3	8	80	13	76.5	0.639
MAP	9	69.2	18	64.3	0.756	9	60	18	69.2	0.548	9	64.3	7	70	11	64.7	0.95
DIA	6	46.2	12	42.9	0.843	5	31.3	13	52	0.192	8	61.5	4	44.4	6	31.6	0.245
DIA + HBP	6	38.5	9	34.6	0.813	2	14.3	12	48	0.035*	5	38.5	4	44.4	5	29.4	0.728
OBS + HBP	9	69.2	14	50	0.248	8	53.3	15	57.7	0.786	8	57.1	5	50	10	58.8	0.901
OBS + DIA	5	38.5	7	25	0.378	3	18.8	9	36	0.236	6	46.2	3	33.3	3	15.8	0.171
OBS + DIA + HBP	4	30.8	5	19.2	0.42	1	7.1	8	32	0.077**	4	30.8	3	33.3	2	11.8	0.334

Table 3: Distribution of CRFs based on Gender, Age and ACE Genotype.
*p-value – Significant (<0.05), **p-value –Borderline Significant
CRFs: Cardiovascular Risk Factors, BMI: Body mass index, HBP: High Blood Pressure, MAP: Mean Arterial Pressure, DIA: Diabetic, OBS: Obesity.

Discussion

The increasing trend of CRFs is a massive threat to the health and sustainable development of developed and developing countries worldwide. Also, in India, heart diseases became a leading cause of premature deaths and are rapidly increasing. The CRFs that cause heart diseases are primarily metabolic impairments (dyslipidemia, hypertension, tobacco use, hyperglycemia and obesity). Similar to previous trends, the subjects in the present study have a high frequency of all the CRFs. Hypertension and obesity are prevalent in more than 50% population, whereas almost 44% of the population is diabetic. A cross-sectional ICMR-INDIAB study also reported higher CRFs in Chandigarh Union Territory (UT). This study reported a high prevalence of diabetes (13.6%), HTN (26.5%), dyslipidemia (84.15%), general obesity (43.45%), abdominal obesity (30%) in Chandigarh compare to other UTs/states. The high-frequency trend in the present study can be justified because the studied subjects are urban dwellers and urban areas are more prone to developing CRFs. Further, the wealthy states and UTs are at a greater risk of heart diseases with a high prevalence of CRFs.

However, another justification that can be given for this high-frequency trend is that the CRFs individually affect each other. A study demonstrated that obesity is a significant risk factor for hyperglycemia [19]. Obesity upsurges the risk of coronary artery disease through insulin resistance, diabetes, arterial hypertension and dyslipidemia [5]. In 2005 surveyed participants, it was reported that nearly half of adult diabetics are obese [37]. A study by Neupane and colleagues also showed that obesity and diabetes are independently associated with hypertension [9]. A study reported that obesity, independently or linked with other CRFs, poses a higher risk of CVDs [1]. The combination of these factors is also higher in the present study ranging from 23.1%-56.1%.

The genetic predisposition was also not denied in the present population as the distribution of ACE I/D polymorphism is not following Hardy-Weinberg equilibrium (p value=0.0003). This deviation can be linked to the high prevalence of CRFs in the population (more than 50%). Moreover, ACE I/D polymorphism was previously associated with CRFs [38-42]. However, some studies showed no association of ACE I/D polymorphism with CRFs [31,43].

Although we found no significant association of the CRFs with ACE I/D polymorphism, there is a differential trend of genotype-specific prevalence of CRFs. The individuals with II genotype have a high frequency of obesity, diabetes, and both. A previous study reported similar results, where ACE II genotype is a risk for diabetes [42]. However, II genotype was not associated with obesity in another study [28]. In the present study, the individual with the DD genotype has a higher prevalence of obesity and hypertension. Similar results were shown by Sun, et al. [28] where ACE DD genotype is associated with hypertension and obesity. A meta-analysis by Mao, et al. [27] also showed the association of DD genotype with overweight/ obesity. However, the low frequency of individuals with two or more CRFs in the DD genotype gives insight into the D allele beneficial role in these individuals. A study by Kumari, et al. [44] presented that the D allele is associated with reducing ACE levels among the north Indian population, giving a protective effect. The higher frequency of CRFs in individuals with ID genotype may suggest its heterozygous advantage over I allele carrier (II) and D allele carrier (DD) with hypertension and a combination of hypertension, obesity and diabetes.

The age and gender-based disparity in CRFs prevalence pointed out the association of both with CRFs in the present study. Females have a higher frequency of abnormal CRFs than males, which is in concordance with previous studies. Moreover, women with more than one CRF have a higher frequency in the present study. It was reported that the prevalence of overweight and obesity in women was significantly higher than that in men [45]. However, studies have shown more significant increases in the prevalence of hypertension among men compared with women [20,21]. The disparity in gender-specific CRFs distribution may contribute to underlying pathophysiology like hormonal changes, pregnancy-related conditions, and cancer therapies that impact cardiovascular anomalies. It was also reported that estrogen protects premenopausal women against hypertension [15].

Another factor that cannot be ignored in the context of CRFs increase is age. The increasing age has several adverse cardiovascular anomalies and is strongly associated with increased CRFs [12,14,18]. A similar trend of frequency distribution is shown in our study, where individuals above 45 years of age are at more risk of cardiovascular disease than younger age groups. The individual with both Diabetes and Hypertension showed a significant increase in their frequency (48% in older age vs 14.3% in younger age). This prevalence is more than previously reported results on Diabetes [11]. Hypertension is the most common problem in middle and older age, leading to cognitive decline and cardiovascular morbidity and mortality [12-14].

Conclusion

These distribution trends and associations in the present population illustrate that cardiovascular disease risk is very high in Chandigarh, India. The primary reason for this might be the lifestyle activity of the enrolled individual, as they all belong to the urban population. The present study suggests that urbanization / modernization, genetics, increasing age,

and gender disparity may increase CRFs. The most critical point of this study is the alarming/exponential increase in CRFs in Chandigarh. One of the limitations of the present study is its sample size. However, it provides the platform for other investigators to investigate further the underlying mechanism of CRFs increase in a bigger sample size in Chandigarh.

Acknowledgement

We would like to thank the members of the study populations and doctors, for voluntarily taking part in this research work and donating their blood samples and cooperation during data collection. We wish to express our deep gratitude to the Dr. Kamla Singh, Director, IRCC, Panchkula for her kind support and guidance.

Funding

This study was funded by the Origin LIFE healthcare solutions & Research centre, Chandigarh, India.

Availability of Data Materials

Although the study is not a clinical trial, it is a genetic study. All data submitted to the Origin LIFE healthcare solutions & Research centre.

Conflicts of Interest

The authors declare that they have no competing interests.

Consent for Publication

Although the manuscript does not involve the use of live photographs of any of the participants, consent was obtained from them for the data to be published as at the time recruitment into the study.

Ethics Approval and Consent to Participate

Ethical committee clearance was obtained from the respective medical institutions (IRCC, Panchkulka) and written well informed consent was obtained from all participants before they were eligible for recruitment into the study.

Authors' Contributions

AK, YS, DK, AR were involved in data collection and laboratory analysis, screening for gene mutations, ST contributed to screening for gene mutations, preparation of the manuscript, statistical analysis. PP, overall supervised and conceptualized the study. All authors read and approved the final manuscript.

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